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See for title page 1 of the description

Method for examining fluids of biological origin and apparatus therefor

The present invention relates to a method for examining a fluid according to the preamble of claim 1. It relates further to devices for executing the method.

Clinical laboratory tests are routinely performed on the serum or plasma of whole blood. The tests commonly employ a series of reactions which terminate after the generation of chromophores which facilitate detection by spectroscopic measurements. The accuracy of most spectroscopic tests is affected to some extent by in vitro interferences. In vitro interferences arise from the fact that biochemical analysis are performed in the complex matrices that make up biological fluids, e.g. serum, plasma or urine. These fluids contain numerous compounds that either have chemical groups that can react with the test reagents or can have the physical or spectral properties of the target analyte. Further, the chemical composition of body fluids can vary with the nature and the extent of disease processes. In vitro interferences can be classified into two classes: spectral and chemical interference. The most commonly observed interferences are hemolysis, icteria, and lipemia. Some 30 % of samples obtained from clinic or hospitalized patients are hemolyzed, icteric, or lipemic. Main reasons for hemolysis are unskilled blood taking or sample preparation, for icteria the jaunice disease, and for lipemia fat nutrition before blood taking.

The goal of sample quality monitoring is the determination of the interfering substances hemoglobin, bilirubin, and lipid prior to conducting fully automated clinical laboratory tests in order to provide meaningful and accurate test results. If a sample is sufficiently contaminated with

interference substances, the test may either not be conducted or the test result may be flagged to be not reliable. Particularly, such a test is desirable in connection with the use of clinical-chemical analyzers which perform most of the analysis of a sample merely full automatically and without respecting special circumstances as regards individual blood samples.

A method and device for semi-quantitative sample quality monitoring of hemoglobin and bilirubin using multiple wavelength measurements on diluted serum samples has been disclosed in US-4,263,512. The method suffers from non-quantitative determination of the interference concentrations and from the need of specific sample conditioning. Alternative methods are chromatographic or clinical-chemical determination of the interference concentrations. The first suffers from high measurement time and delicate instrumentation, whereas the second is not suited for reagentless measurement.

Therefore, it is an aim of the present invention to provide a method for estimating rapidly the content of at least one component in a biological fluid.

Such a method is given in claim 1, the remaining claims presenting preferred embodiments and applications thereof and an installation for executing the method.

In a preferred method according to the invention, the combination or superposition of the extinction spectrum of this first one of the components in a pure state and a function approximating the background extinction is fitted to the measured spectrum of the fluid to be analyzed in a wavelength range, where the component to be determined shows a significant or characteristic shape of its extinction

curve. The function approximating the background extinction may e.g. be a straight line, and in this case, the wavelength range is preferably chosen where the expected background extinction spectrum is similar to a straight line.

The invention shall be further explained in referring to exemplary embodiments with reference to the Figure:

Fig. 1: Schematic representation of a photometric spectrum measurement arrangement.

Fig. 2: Extinction spectra of pure components and a standard blood serum.

Fig. 3: Normalized extinction spectra of real whole blood sera, bilirubin and hemoglobin contribution being subtracted, and a reference lipid solution sample.

Fig. 4: Evaluation method for sample quality monitoring.

Fig. 5: Experimentally measured extinction spectrum of a real whole blood serum and results of the evaluation method.

Fig. 6: Measured extinction spectrum of a strongly hemolytic whole blood sample and respective extinction spectra obtained by the examination method.

Fig. 7: As Fig. 6 for a strongly icteric whole blood sample.

Figs. 8, 9: Optically determined hemoglobin (Fig. 8) and bilirubin (Fig. 9) concentrations versus added concentrations for 125 independent test samples.

Figs. 10,11: Optically measured hemoglobin respectively bilirubin concentration values vs. clinical-chemically measured concentration values for independent real whole blood sera.

Figs. 12-15: Optically measured hemoglobin and bilirubin concentrations of 92 real whole blood sera and the respective CV values obtained using a state-of-the-art spectrometer.

Fig. 16, 17: Low-cost versus state-of-the-art spectroscopically measured (Fig. 16) hemoglobin and bilirubin (Fig. 17) concentrations of 92 real whole blood sera.

Fig. 18: Schematic illustration of a dip probe.

Sample quality monitoring of blood serum or plasma by optical absorption spectroscopy in the visible and near IR range is investigated. The target measuring ranges are 0.1 - 10 g/l hemoglobin, 2 - 20 mg/dl (1 dl = 0.1 liter) bilirubin and 100 - 2000 mg/dl lipid with a measurement accuracy of 20 %. The evaluation is performed by the method according to the present invention, yielding the content of the hemoglobin and bilirubin. Lack of a reproducible relation

between light-scattering and lipid concentration inhibits quantitative determination of the lipid concentration by optical absorption spectroscopy. Therefore, it is provided a differential extinction spectrum, which is obtained from subtracting the hemoglobin and bilirubin contributions from the extinction spectrum of the target sample. It contains the spectral contributions of the lipid and the matrix, e.g. the blood serum or plasma, which can then be investigated for spectral anomalies. The method is experimentally investigated using a series of 125 synthetic test samples and a series of 92 real blood sera. Accuracy and reproducibility of the technique versus the performance of the spectroscopic measurement device are analyzed.

The basic setup for optical absorption spectroscopy for sample quality monitoring is shown in Fig. 1. The beam 1 of a multiple optical wavelength light source 2 is collimated by lens 3, which directs the light of spectral intensity $I_0(\lambda)$ to the target sample. The optical path in the target sample is denoted by d. Lens 4 collects the transmitted light of intensity $I(\lambda)$, which is then detected by a spectral wavelength analyzer, symbolized by its input 5.

Optical absorption is commonly characterized by the extinction $E(\lambda)$, which is defined as

$$\frac{I(\lambda)}{I_0(\lambda)} = 10^{-E(\lambda)} . \qquad (1)$$

In the presence of J interfering substances in the target sample, e.g. hemoglobin, bilirubin and lipid (i. e., J=3), the extinction can be described by the linear combination

$$E(\lambda) = \sum_{j=1}^{J} K_j(\lambda) \frac{d}{q_{dil}} C_j + E_g(\lambda) , \qquad (2)$$

where K_j and C_j are the specific extinction coefficient and the concentration of interfering substance j (j = 1, 2, ..., J), respectively. The dilution of the target sample is denoted by q_{dil}, i. e. (original concentrations):(sample concentrations)=(1:q_{dil}). E_g is the extinction characteristic of the matrix, e.g. blood serum or plasma. Figure 2 shows the extinction coefficients K_h 10 of hemoglobin, K_b 11 of bilirubin, and K_l 12 of lipid (Intralipid 20% [Pharmacia, Sweden]) in the visible and near IR range. The extinction spectrum E_g 13 of a standard blood serum (Control Serum N (human) [Hoffmann-La Roche, Switzerland]) is also shown in Fig. 2 (dashed line).

In the scope of sample quality monitoring, a minimum number of N_{min} = 4 statistically independent extinction values $E(\lambda_n)$ (n = 1, ..., 4) should allow to determine the four unknown parameters in Eq. (2), i.e. the concentration of the interference substances hemoglobin (C_h), bilirubin (C_b) and lipid (C_l), and the matrix part (E_g). More reproducible results are expected by least squares fitting the model of the extinction spectrum $E(\lambda)$ in Eq. (2) to $N > N_{min}$ measured values $E(\lambda_n)$ (n = 1, 2, ..., N) in order to obtain best estimates of the values of C_h, C_b and C_l.

However, it is observed that the specific extinction coefficient $K_l(\lambda)$ of lipid is not reproducible in real blood sera, which is mainly due to the statistical distribution of the size of the scattering centers in the lipid. Further, the monotonically decreasing extinction spectrum of lipid versus wavelength lacks typical (local) characteristics (Fig. 2). Therefore, it cannot be distinguished from the extinction spectrum of the matrix (E_g).

Figure 3 shows extinction spectra of real whole blood sera, from which the hemoglobin and bilirubin contributions have

been subtracted. These differential spectra therefore represent the sum of the spectral contributions of the lipid and the matrix. The shown extinction spectra are normalized to the extinction at λ = 700 nm. The solid line 30 refers to the reference solution of Intralipid, the broken lines 31 refer to several samples of real whole blood sera after subtraction of the extinction contribution by hemoglobin and bilirubin.

Hence quantitative determination of the hemoglobin (C_h), bilirubin (C_b) and lipid (C_l) concentrations appears not to be possible by measuring the optical extinction spectrum of the target sample and fitting the model in Eq. (2) to the measured values $E(\lambda_n)$.

Therefore, sequential determination of first the hemoglobin (C_h) and then the bilirubin (C_b) concentration is proposed. The differential spectrum E_{diff} is obtained from subtracting the hemoglobin and bilirubin contributions from the measured extinction spectrum. E_{diff} represents the sum of the spectral contributions of the lipid (E_l) and the matrix (E_g), and may additionally be investigated for spectral anomalies over the whole spectral range.

The method (cf. Fig. 4) is based on approximating the differential spectrum $E_{diff} = E_g + E_l$ in a limited wavelength range λ_r by a straight line. First, the hemoglobin concentration is determined 38 from the measured extinction spectrum $E(\lambda)$ 35 in the approximate wavelength range $\lambda_{rh} \cong$ [545, 575] nm, where the hemoglobin has typical spectral characteristics and the bilirubin contribution is quasi negligible (Fig. 2). The extinction spectrum is approximated by

$$E_l(\lambda) = E_d(\lambda) + E_h(\lambda) \ , \qquad (3)$$

where

$$E_h(\lambda) = K_h(\lambda) \frac{d}{q_{dil}} C_h \qquad (4)$$

is the hemoglobin contribution and $E_d = a_{0h} + a_{1h}\lambda$ linearly approximates the sum of the lipid and the matrix contributions. Note that the parameters a_{0h} and a_{1h} have no physical significance. Fitting 37 the model of the extinction spectrum in Eqs. (3) and (4) by a linear least squares algorithm to N_1 spectroscopically measured values $E(\lambda_n)$ ($n = 1, 2, \ldots, N_1$) in the range λ_{rh} then delivers the best estimate of the hemoglobin concentration C_h (and a_{0h}, a_{1h}) 38.

Then, the bilirubin concentration is determined from the measured extinction spectrum $E(\lambda)$ 35 in the wavelength range $\lambda_{rb} \cong [480, 545]$ nm. The extinction spectrum is approximated by

$$E_2(\lambda) = E_h(\lambda) + E_d(\lambda) + E_b(\lambda) , \qquad (5)$$

where

$$E_b(\lambda) = K_b(\lambda) \frac{d}{q_{dil}} C_b \qquad (6)$$

is the bilirubin contribution, E_h is the previously determined hemoglobin contribution, and $E_d = a_{0b} + a_{1b}\lambda$ linearly approximates the sum of the lipid and the matrix contributions. Fitting 41 the model of the extinction spectrum in Eqs. (5) and (6) by a linear least squares algorithm to N_2 spectroscopically measured values $E(\lambda_n)$ ($n = 1, 2, \ldots, N_2$) in the wavelength range λ_{rb} then delivers the best estimate of the bilirubin concentration C_b (and a_{0b}, a_{1b})

42. Finally, using Eqs. (2), (4) and (6), the differential spectrum E_{diff} 45 is obtained by

$$E_{diff}(\lambda) = E(\lambda) - E_h(\lambda) - E_b(\lambda) \ , \qquad (7)$$

which can then be investigated for spectral anomalies 46.

Reproducibility

The reproducibility of the measured concentrations C_h and C_b of hemoglobin respectively bilirubin can be analytically calculated, if the minimum number $N_{min} = 3$ of measured extinction values $E(\lambda_n)$ in the range $\lambda_{rh[b]}$ are used to determine c_h [c_b] from Eq. (3) respectively Eq. (5). The reproducibility of a measured concentration C is commonly characterized by the coefficient of variation $CV = \sigma_c / E\{c\}$, where σ_x and $E\{x\}$ stand for the standard deviation and the statistical expectation (mean value) of $\{x\}$, respectively. Using Eqs. (1), (2), and (7), it can be readily shown that CV of the concentration C_j is related to the reproducibility of the (physically) measured optical intensity σ_I/I_0 through

$$CV\big|_{N_{min}} = \frac{\sigma_{C_j}}{E\{C_j\}} \cong \frac{1}{D}\frac{\sigma_I}{I_0}\frac{1}{C_j}\,10^{K_j(\bar{\lambda})\frac{d}{q_{dil}}C_j + E_d(\bar{\lambda})} \ , \qquad (8)$$

where $\bar{\lambda}$ 47 is the center wavelength of the respective measurement range λ_r, $D = [\ln(10)/4]\cdot[2K_j(\lambda_2) - K_j(\lambda_1) - K_j(\lambda_3)]\cdot[d/q_{dil}]$, and $\ln(x)$ is the natural logarithm of (x). Note that the background contribution $E_d(\lambda)$ of the lipid and the matrix significantly reduce the reproducibility of the measured concentration C_j.

When $N > N_{min}$ statistically independent measured values $E(\lambda_n)$ are used for the linear least squares algorithm, it can be

shown that the CV 48 [49] of the measured hemoglobin [bilirubin] concentration is related to Eq. (8) through

$$CV|_N = \frac{1}{\sqrt{M}} CV|_{N_{min}}, \qquad (9)$$

where $M = N - N_{min}$ is the number of redundant measurements. Hence the reproducibility of the measured concentration increases with the number N of measured extinction values considered for the fit procedure. The number N is given by the spectral resolution and sampling rate of the spectroscopic measurement system and the wavelength range λ_r. Note that extension of λ_r increases N for a given spectral resolution and sampling rate, but the linear approximation E_d of the sum of the lipid and the matrix contributions in Eqs. (3) and (5) becomes more and more inaccurate. The value of CV, calculated from Eqs. (8) and (9), can then be compared 50 with a predetermined limiting value CV_{lim} in order to characterize the quality of the concentration measurement: A value of CV exceeding CV_{lim} signifies a critical to weak reproducibility 51 of the results, i. e. concentrations and differential spectrum. Consequently, the measurement would e.g. be disregarded, repeated, or assigned reduced reliability.

The bloc diagram in Fig. 4 summarizes the proposed measurement and evaluation method for sample quality monitoring.

Sample quality monitoring, based on optical absorption spectroscopy as shown in Fig. 1, has been experimentally investigated using a state-of-the-art spectrometer (Cary V, VARIAN, Australia). The collimated beam had an approximate spot size of 5 x 2 mm^2. The optical path in the test sample was d = 10 mm. The sample dilution was 1:20 (q_{dil} = 20). The spectrum of the test sample was measured in the wavelength

range λ = [300, 1200] nm with a spectral resolution of $\Delta\lambda$ = 0.05 nm and a spectral sampling rate of $\Delta\lambda_s$ = 1 nm/pixel. The hemoglobin concentration C_h has been obtained from linear least squares fitting the model in Eqs. (3) and (4) to N_1 = 28 measured values $E(\lambda_n)$ in the wavelength range λ_{rh} = [545, 575] nm. The bilirubin concentration C_b has been obtained from linear least squares fitting the model in Eqs. (5) and (6) to N_2 = 63 measured values $E(\lambda_n)$ in the wavelength range λ_{rb} = [480, 545] nm. The differential extinction spectrum E_{diff} has been obtained from Eq. (7). The reproducibility of the measured hemoglobin and bilirubin concentrations has then been calculated according to Eqs. (8) and (9), with $\sigma_I/I_0 = 5\cdot10^{-5}$ for the reproducibility of the measured optical intensities.

As an example, Figure 5 shows the experimentally measured extinction spectrum $E(\lambda)$ of a typical real whole blood serum 55. The best fitting extinction models for hemoglobin 57 and bilirubin 59 in Eqs. (3) and (5) are represented by crosses and dots, respectively. The best fitting hemoglobin and bilirubin concentrations are C_h = 0.18 g/l (CV = 1.5 %) and C_b = 0.67 mg/dl (CV = 0.3 %), respectively. The differential extinction spectrum $E_{diff}(\lambda_n)$ 60 is also shown by the dashed line.

Figs. 6 and 7 show other examples of real whole blood serum samples, namely with a high hemoglobin content respectively an highly icteric sample. Furthermore, in Fig. 6, the differential spectrum shows an anomalous differential spectrum which is merely constant with additionally an increased extinction with increasing wavelength above about 650 nm. The continuous line 62 is the measured spectrum, the dashed line 64 and the dotted line 65 are the hemoglobin respectively the bilirubin contributions, and the dash-

dotted line 66 is the differential spectrum, each time calculated from the results according to the described method.

Accuracy

In order to investigate the accuracy of the method, the hemoglobin and bilirubin concentrations of a series of 125 independent test samples have been determined. The samples have been synthesized using a standard blood serum (Control Serum N (human) [Hoffmann-La Roche, Switzerland]) to which hemoglobin (Hemolysat [Hoffmann-La Roche, Switzerland]), bilirubin (B-4126 mixed isomers [Sigma, Switzerland]) and lipid (Intralipid 20% [Pharmacia, Sweden]) have been added. The added concentrations of hemoglobin, bilirubin and lipid were C_h = [0, 0.17, 0.83, 3.33, 15] g/l, C_b = [0, 1, 2, 10, 20] mg/dl and C_l = [0 50 100 400 1800] mg/dl, respectively, leading to the set of 5·5·5 test samples. The optically measured hemoglobin 70 and bilirubin 72 concentrations versus added concentrations are represented in Fig. 8 and 9.

In the case of hemoglobin (Fig. 8), a linear least squares fit 74 ($C_{fit,h} = C_{0,h} + m_h C_h$) yields an offset concentration $C_{0,h}$ = 0.12 g/l and a slope m_h = 0.95. The correlation coefficient between the best fit and the measured values is ρ = 0.999. In the case of bilirubin (Fig. 9), a second linear least squares fit 76 ($C_{fit,b} = C_{0,b} + m_b C_b$) yields an offset concentration $C_{0,b}$ = 1.64 mg/dl and a slope m_b = 0.999. The correlation coefficient between the best fit and the measured values is ρ = 0.995. Note that Control Serum N (human) has an approximate bilirubin concentration of $C_b \cong 2$ mg/dl. Further, it is stated that the amount of added hemoglobin, bilirubin and lipid also has finite accuracy.

The hemoglobin and bilirubin concentrations of a series of 92 real whole blood sera have then been optically determined. The concentration values were in the range C_h = [0, 5] g/l for hemoglobin and C_b = [0, 45] mg/dl for bilirubin. As reference values, the concentrations have been determined by clinical-chemical analysis(Cobas® Integra 700 analyzer, [Hoffmann-La Roche, Switzerland]). Figures 10 and 11 show the optically versus clinical-chemically determined hemoglobin [bilirubin] concentrations 90 [91].

The results show that the sensitivity of the method is approximately $C_{h,min} \cong 0.5$ g/l hemoglobin and $C_{b,min} \cong 2$ mg/dl bilirubin. The observed correlation coefficients between the reference 93 [94] and the optically determined 90 [91] hemoglobin [bilirubin] concentrations were $\rho = 0.980$ and $\rho = 0.996$, respectively. Note that the clinical-chemical method has also limited accuracy; namely the bilirubin concentrations (Fig. 11) show better correlation than the hemoglobin concentrations (Fig. 10), although the accuracy of the optically measured bilirubin concentration is affected by the accuracy of the hemoglobin concentration determination (sequential determination of hemoglobin and bilirubin, see above).

In comparison, the benchmark Hitachi-Formula (US-4,263,512) evaluation algorithm has been used to evaluate the optical absorption spectra. The observed correlation coefficients between the reference and the Hitachi-Formula determined concentration values were $\rho = 0.879$ for hemoglobin and $\rho = 0.992$ for bilirubin.

Reproducibility

The coefficient of variation CV of the measured hemoglobin and bilirubin concentrations has been calculated from Eq.

(8), where $\sigma_I/I_0 = 5 \cdot 10^{-5}$ was the reproducibility of the measured optical intensities and $\bar{\lambda} = 560$ nm [$\bar{\lambda} = 512$ nm] was the center wavelength of $\lambda_{rh[b]}$. Figures 12 to 15 show the optically measured hemoglobin (Fig. 12) and bilirubin (Fig. 14) concentrations of the set of 92 real blood sera and the respective CV values (Fig. 13 resp. Fig. 15). Inspection of Figs. 12 to 15 shows that the reproducibility is better for large concentration values, and that the values for hemoglobin and bilirubin are better than CV < 10 % respectively < 1 % for 89 respectively 91 of 92 analyzed sera.

Low cost optical spectrometer setup

Sample quality monitoring, based on optical absorption spectroscopy as shown in Fig. 1, has then been experimentally investigated with low cost optical elements. The multiple optical wavelength light source was a white-light halogen lamp (Halogen 5V, 5W, $P_v \cong 2$ nW/nm @ $\lambda = 530$ nm [MICROPARTS GmbH, Germany]). The collimated beam had an approximate diameter of D = 2 mm. The optical path in the test sample was d = 10 mm. The dilution of the sample was 1 : 20 ($q_{dil} = 20$). The transmitted light was collected by a lens (focal length f = 5 mm) and coupled into an optical fiber with core diameter $\varnothing_c = 100$ µm. The light was spectroscopically analyzed by a low cost, plane-concave spectrometer PCS [CSEM-Z, Switzerland] with spectral resolution $\Delta\lambda \cong 8$ nm. The spectrum of the test sample was measured by a linear photodiode array (512 pixels, center-to-center spacing $\Delta x = 25$ µm) in the wavelength range $\lambda =$ [421, 704] nm. The spectral sampling rate was $\Delta\lambda_s = 2.8$ nm/pixel. The reproducibility of the measured optical intensities was $\sigma_I/I_0 = 5 \cdot 10^{-4}$. The hemoglobin concentration C_h has been obtained from linear least squares fitting the model in Eqs. (3) and (4) to $N_1 = 11$ measured values $E(\lambda_n)$ in

the wavelength range λ_{rh} = [545, 575] nm. The bilirubin concentration C_b has been obtained from linear least squares fitting the model in Eqs. (5) and (6) to N_2 = 20 measured values $E(\lambda_n)$ in the wavelength range λ_{rb} = [480, 545] nm. The differential extinction spectrum E_{diff} has been obtained from Eq. (7). Figures 16 and 17 show the PCS versus the state-of-the-art (Cary V) spectroscopically measured hemoglobin respectively bilirubin concentrations of the set of 92 blood sera of Figs. 10, 11 and 12 to 15. In the case of hemoglobin (Fig. 16), a linear least squares fit ($c_{fit,h} = c_{0,h} + m_h C_h$) in the concentration range $C_h < 2$ g/l yields an offset concentration $c_{0,h}$ = 0.043 g/l and a slope m_h = 0.859. The correlation coefficient between the best fitting curve and the PCS measured values is ρ = 0.997. In the case of bilirubin (Fig. 17), a linear least squares fit ($c_{fit,b} = c_{0,b} + m_b C_b$) in the concentration range $C_b < 15$ mg/dl yields an offset concentration $c_{0,b}$ = -0.010 mg/dl and a slope m_b = 0.940. The correlation coefficient between the best fitting curve and the PCS measured values is ρ = 0.998. The results show that low cost spectrometers can readily be used for sample quality monitoring purposes.

If the examination of the samples yields a result indicating an anomalous condition of the sample, there may be generated by the examining device, e. g., one or more of the following signals or responses:

- an optical and/or acoustical warning signal to excite the operator's attention, particularly in case an abnormal sample has been detected,
- a printout of results (spectra, coefficients etc.) on a printer,
- a print on the analyzer's printout, so that the operator can immediately see if the results of the regular, chemical-clinical examination are true or prone to artefacts, or

- an automatic repetition of the measurement, e.g. using a new test sample.

The described method may be implemented in various arrangements, preferably in connection with an automated analyzer, e g. as follows:

- The quality test may be done as a first photometric pass in the photometric site of an analyzer. Thereby, the performance of the analyzer is reduced because this prescan and the regular photometric pass are performed subsequently, or an additional sample is needed causing consumption of sample material;
- An additional photometric site is provided for the quality test;
- The pipette, or more generally, the supply system of the analyzer for the fluids to be tested, is provided with a transparent site, i. e. an optical flow-through cell (OFTC), in connection with a photometer; where necessary, particularly when the conduit system subsequently provides differently diluted samples, there may be arranged different OFTC paths with different optical path lengths in connection with flow switches for compensating the varying dilutions;
- A stand-alone photometer dedicated to the quality tests
- A probe 110 for immersing into a sample container 111 as exemplary shown in Fig. 11: At its end 112, it is provided with a lateral recess 113 serving as the optical path. The light 115 passes the recess 114 within the probe shaft and is reflected back by a prism 116 so that it traverses the recess 113. Above the recess, by an appropriate optic 117, the light having passed is collected and forwarded to the photometer (not shown) by a light guide 119.

- The sample tube itself may be used as the photometric cuvette, provided the differing lengths of the optical observation paths can be compensated for, i. e. the path lengths are determined and can be input into the quality test system, and/or the sample tubes are of sufficiently equal size so that the optical paths do only differ within small limits, maybe in an even negligible variation range.

From the above description, variations of the invention are conceivable to the one skilled in the art without leaving the scope of the invention as defined in the claims. For instance, it is conceivable:

- to extend the method to the determination of a third and further components by continuing the sequential determination method using two, three, four etc. previously determined components for ascertaining the concentrations of a third, fourth etc. component;
- to have the differential spectrum analyzed automatically by determining its curvature (i. e. the second derivative) and/or slope (i. e. the first derivative), which should increase respectively be negative for increasing wavelength in the exemplary quality test set forth above;
- to choose deviating wavelength ranges for the photometric measurements, particularly if the quality test is used for determining other components of the samples provided that the spectra to be combined in order to approximate the measured spectrum show peculiarities in the given wavelength range so that the approximation parameters, before all the concentration of the sought component, are unambiguously derivable;
- to determine the differential spectrum in a subrange of the wavelength range used for the determination of the

single components, or possibly even a range extending beyond this range.

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Claims

1. Method for evaluating an extinction spectrum of a fluid of preferably biological origin, with at least two components, characterized in that a first extinction spectrum $E_1(\lambda)$ of the fluid is measured in a first wavelength range $\lambda = \lambda_{1,1}$ to $\lambda_{1,n}$, and an approximated spectrum $\overline{E}_1(\lambda)$ is fitted to this extinction spectrum, the approximated spectrum being a combination, preferably the sum, of

- a predetermined approximation function $f_1(\lambda, a_{i,1})$, with i ranging from zero to at least one, for the background extinction, and
- the predetermined extinction spectrum $E_1(c_1, \lambda)$ of a pure first component of concentration c_1 of the components to be determined,

the fitting being performed by varying the concentration c_1 and at least two of the coefficients $a_{i,1}$, so that the deviation between measured spectrum and approximated spectrum is minimized, in order to determine the concentration of the first component wherein the wavelength ranges are selected such that the concentration c_1 of the component can be determined unambiguously.

2. A method according to claim 1, characterized in that in at least one further second wavelength range $\lambda = \lambda_{k,1}$ to $\lambda_{k,n}$, $k \geq 2$, a second extinction spectrum $E_k(\lambda)$ of the fluid is measured and a second approximated spectrum $\overline{E}_k(\lambda)$ is fitted to the second spectrum, the second approximated spectrum $\overline{E}_k(\lambda)$ being a combination, preferably the sum of:

- a predetermined approximation function $f_k(\lambda, a_{i,k})$ with i ranging from zero to at least one, for the background extinction,

- the combination, particularly the sum, of the extinctions $E_l(c_l,\lambda)$, l=1 to k-1, of the k-1 pure first and second components priorly determined, and
- the predetermined extinction $E_k(c_k,\lambda)$ of the said pure second component k of concentration c_k to be determined,

the fitting being performed by varying the concentration c_k and at least two of the coefficients $a_{i,k}$ so that the deviation between measured spectrum and approximated spectrum is minimized, in order to determine the concentration of the second component, wherein the wavelength ranges are selected such that the concentration c_k of the said second component k can be determined unambiguously.

3. A method according to claim 1 or 2, characterized in that at least one, preferably all, of the functions $f_k(\lambda,a_{i,k})$, $k \geq 1$, are of the form $f_k(\lambda,a_{i,k}) = \sum_{i=0}^{n} a_{i,k}\,\lambda^i$, with $n \geq 1$ and preferably n=1.

4. A method according to one of claims 1 to 3, characterized in that the fit of the approximated spectra $\bar{E}_k(\lambda)$, $k \geq 1$, to the measured values $E(\lambda_i)$, with i=1 to N, N being the number of measured values, is done by a least squares fit.

5. A method according to one of claims 1 to 4, characterized in that the sample is marked at least anomalous if the determined concentrations C_k, $k \geq 1$, are outside a predetermined range.

6. A method according to one of claims 1 to 5, characterized in that a differential spectrum $E_{diff}(\lambda) = E(\lambda) - \sum_{j=1}^{J} \bar{E}_j(C_j,\lambda)$, with J being the number of components, and λ being in a range covering at least 30 %, preferably at least

50 % and most preferably about 100 % or more of the whole wavelength range defined by the broadest combination of $\lambda_{1,1}$ and $\lambda_{1,n}$, $\lambda_{2,1}$ and $\lambda_{2,n}$, ..., $\lambda_{J,1}$ and $\lambda_{J,n}$ is computed, and the differential spectrum is subjected to an analysis in view of anomalies.

7. A method according to claim 6, characterized in that the curvature and/or the slope of the differential spectrum in at least one predetermined wavelength range is/are determined, the result compared with the expected values, and in that the differential spectrum is estimated to be normal if the values compared have identical sign, optionally with the magnitude resting in a predetermined range given by an upper and a lower limiting curve.

8. A method according to one of claims 1 to 7, characterized in that
the sample is blood, preferably human blood, or a fluid derived therefrom,
the first wavelength range is chosen in the range of 500 to 600 nm, preferably from 545 nm to 575 nm, even more preferably being essentially identical with one of these ranges, the first reference spectrum $E_1(\lambda)$ being that of hemoglobin, so that the concentration C_1 of hemoglobin is determinable, and
the second wavelength range is chosen in the range of 400 to 600 nm, preferably from 480 nm to 545 nm, even more preferably being essentially identical with these ranges, the second reference spectrum $E_2(\lambda)$ being that of bilirubin, so that the concentration C_2 of bilirubin is determinable.

9. A method according to claim 8, characterized in that the lipid concentration and the overall constitution of the sample are estimated to be normal if the differential spectrum has a negative slope and/or a positive curvature.

10. A method according to one of claims 8 to 9, characterized in that the sample is estimated to be of critical condition if the concentration of bilirubin and/or hemoglobin exceed a predetermined value, and/or if the differential spectrum is anomalous.

11. A method according to one of claims 1 to 10, characterized in that the spectra are provided as electrical signals and furnished to an evaluation device comprising a processor which performs the method steps on the spectra under the control of a program, and that the results are stored in a storage means, preferably a storage means for digital data, and/or presented to an operator, preferably by printing, displaying and/or producing audible sounds.

12. An installation for implementing the method of one of claims 1 to 11 for use with an analyzer, preferably a clinical-chemical analyzer, characterized in that in the supply path of sample fluid of the analyzer, at least one photometric measurement site is provided so that extinction spectra can be taken of the fluid in the supply path.

13. A photometric probe for implementing the method of one of claims 1 to 11, characterized in that its end comprises a photometric measurement site confined by two facing walls, one of which being equipped with a light source, and the second well being equipped with a light capturing means, the measurement site, the light source and the light capturing means being so arranged that light emanating from the light source passes the measurement site and, at least to a significant part, is captured by the light capturing means.

14. A photometric probe according to claim 13, characterized in that it comprises a light guide passing the measurement

site, and that a light deviating means, preferably a prism, is arranged such that light exiting the light guide is deviated, preferably by an angle of substantially 180°, towards the light exiting side of the first wall of the measurement site.

15. An analyzer, preferably a chemical-clinical analyzer, with an installation for photometric measurements, characterized in that the installation comprises a program memory and a device for executing the program, wherein the execution of the program implements the method of one of claims 1 to 11.

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Abstract

A quality test of fluids of biological origin can be performed optically with applying a suitable evaluation method. In case of two components to be determined in a such fluid, an extinction spectrum is approximated in a first wavelength range by a combination of a merely theoretical curve and the spectrum of the pure first substance in a first wavelength range, and this evaluation is repeated in a second wavelength range this time by approximating the measured spectrum (62) by a combination of a hypothetical curve, the spectrum (64) of the first component with the already determined concentration, and the spectrum (65) of the pure second component. Furthermore, it is feasible to subtract the first and second spectrum and analyze the so obtained differential spectrum (66) in view of anomalies. The hypothetical curves are preferably straight lines which are defined by slope and ordinate section. In the praxis of the quality test of blood, bilirubin and hemoglobin may be quantitatively be determined, whilst the background together with the lipid component can be qualitatively examined by means of the differential spectrum.

- - - - -

(Fig. 6)

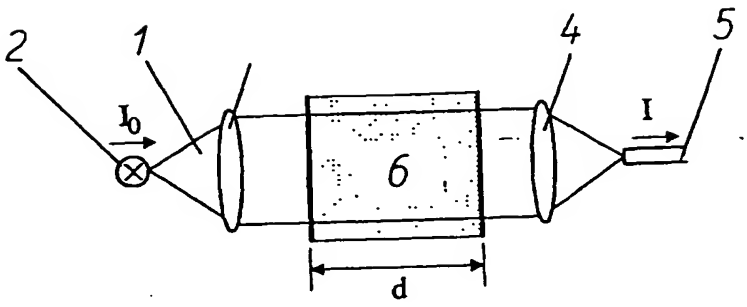
2
1
4
5
I_0
I
6
d

Fig. 1

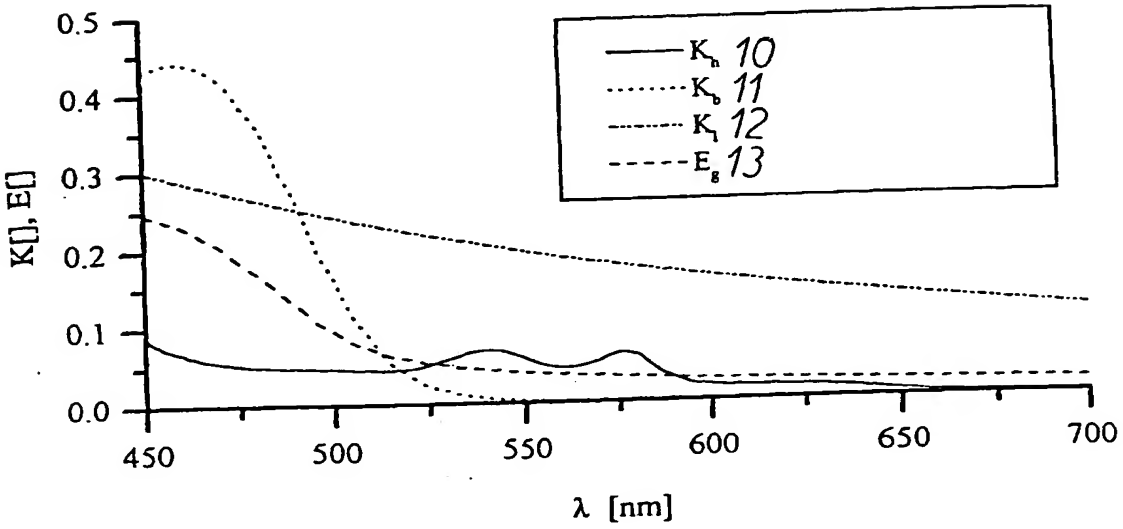
0.5
0.4
0.3
0.2
0.1
0.0
K[], E[]
450
500
550
600
650
700
λ [nm]
K_h 10
K_b 11
K_t 12
E_g 13

Fig. 2

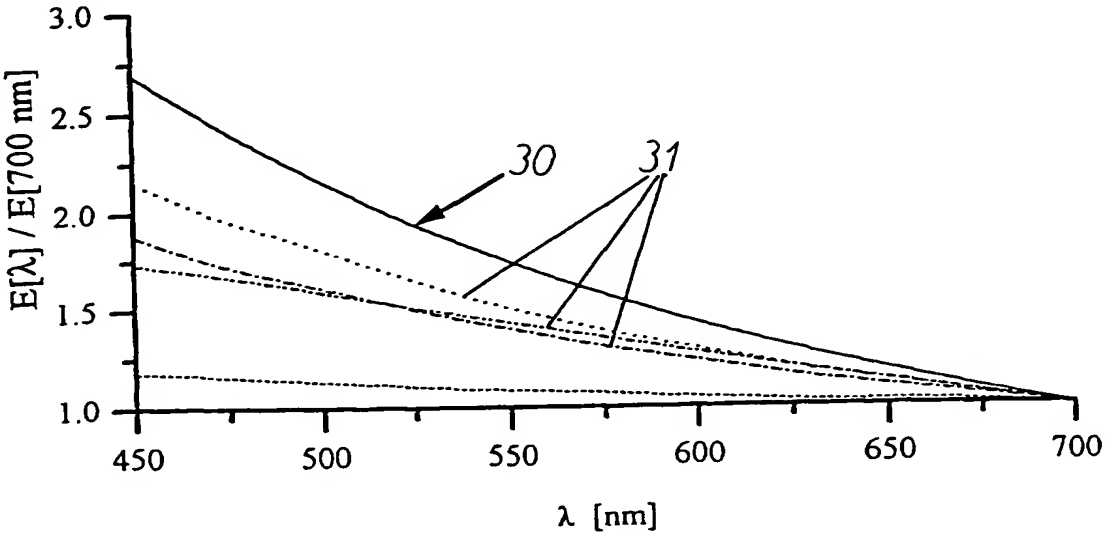
3.0
2.5
2.0
1.5
1.0
E[λ] / E[700 nm]
30
31
450
500
550
600
650
700
λ [nm]

Fig. 3

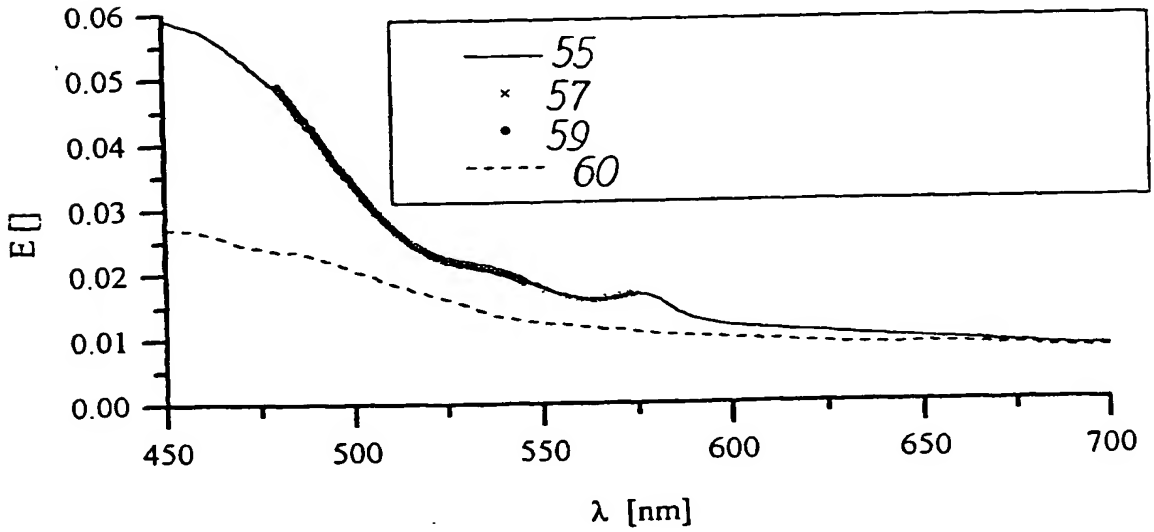
0.06
0.05
0.04
0.03
0.02
0.01
0.00
E[]
55
57
59
60
450
500
550
600
650
700
λ [nm]

Fig. 5

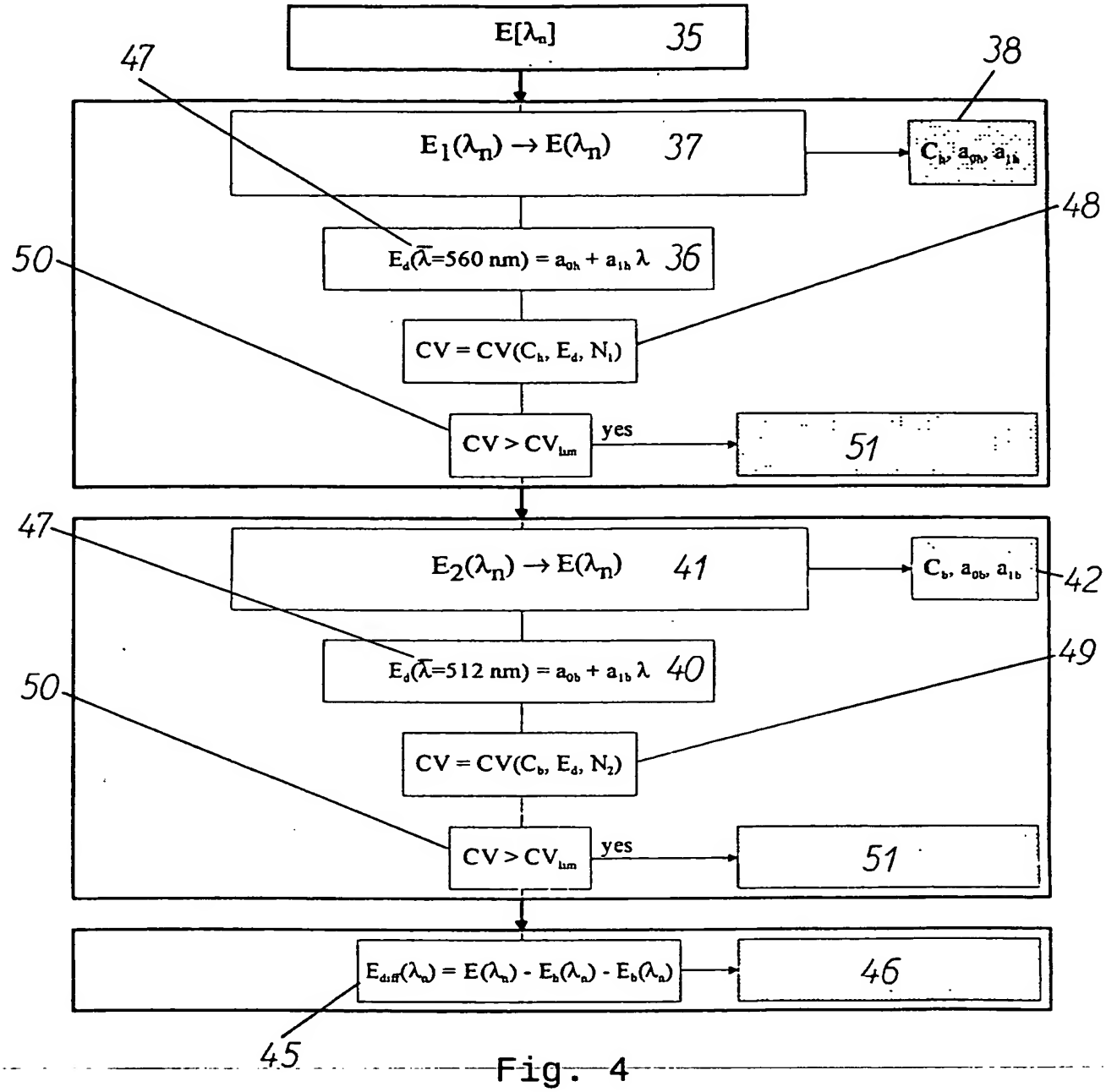
E[λn] 35
47
38
E1(λn) → E(λn) 37
Cb, a0b, a1b
48
Ed(λ̄=560 nm) = a0b + a1b λ 36
50
CV = CV(Cb, Ed, N1)
CV > CVlim
yes
51
47
E2(λn) → E(λn) 41
Cb, a0b, a1b
42
Ed(λ̄=512 nm) = a0b + a1b λ 40
49
50
CV = CV(Cb, Ed, N2)
CV > CVlim
yes
51
Ediff(λn) = E(λn) - Eb(λn) - Eb(λn)
46
45

Fig. 4

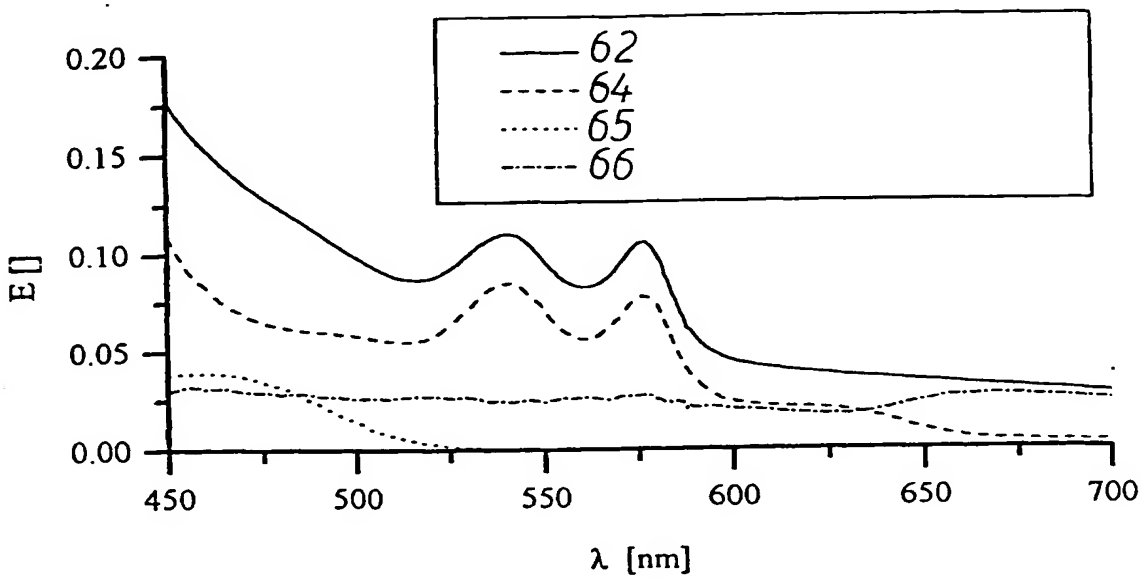
62
64
65
66
0.20
0.15
0.10
0.05
0.00
E[]
450
500
550
600
650
700
λ [nm]

Fig. 6

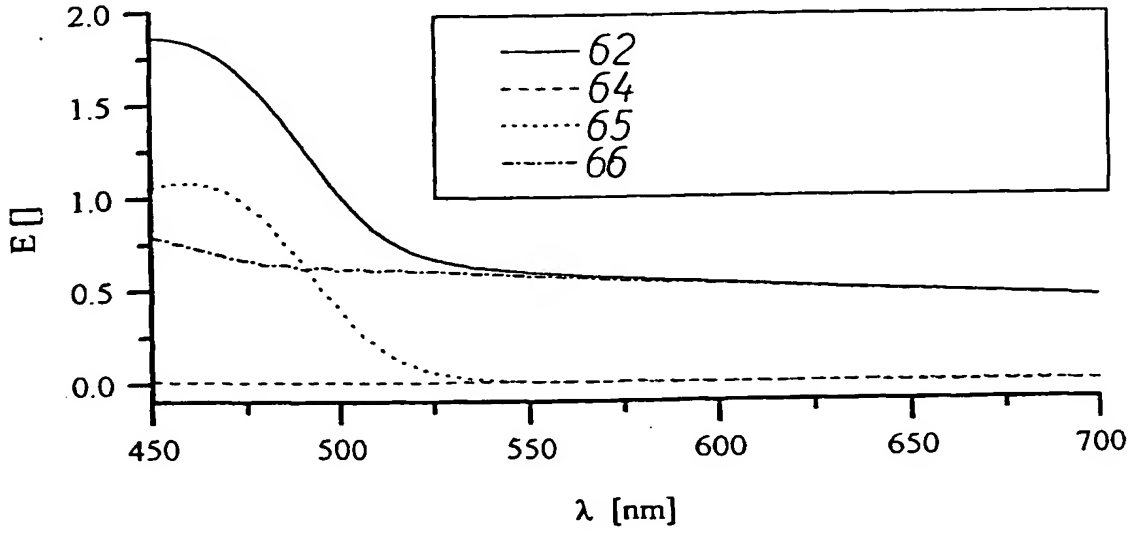
62
64
65
66
2.0
1.5
1.0
0.5
0.0
E[]
450
500
550
600
650
700
λ [nm]

Fig. 7

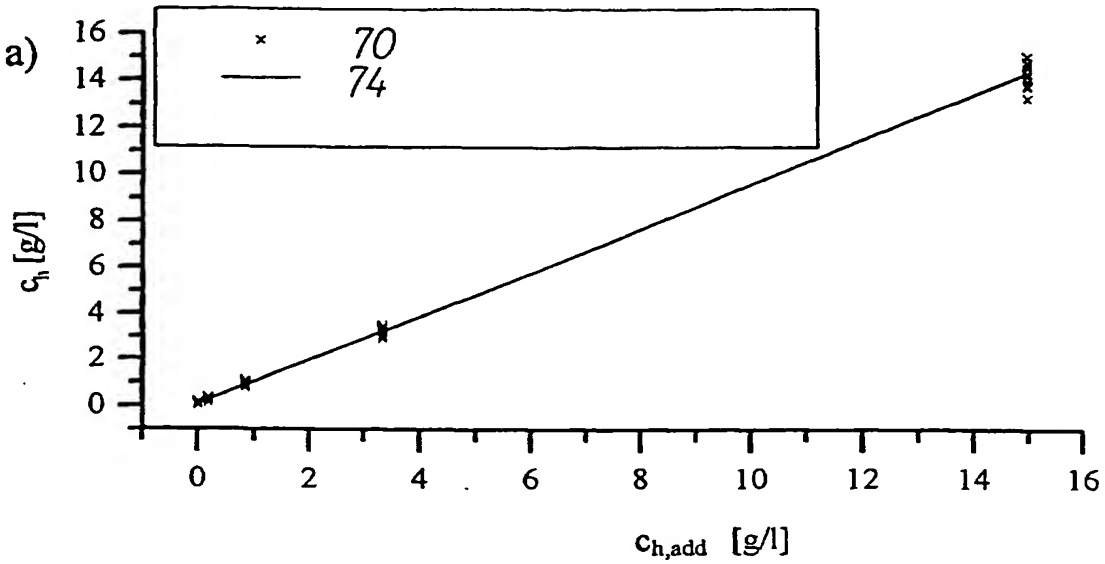

Fig. 8

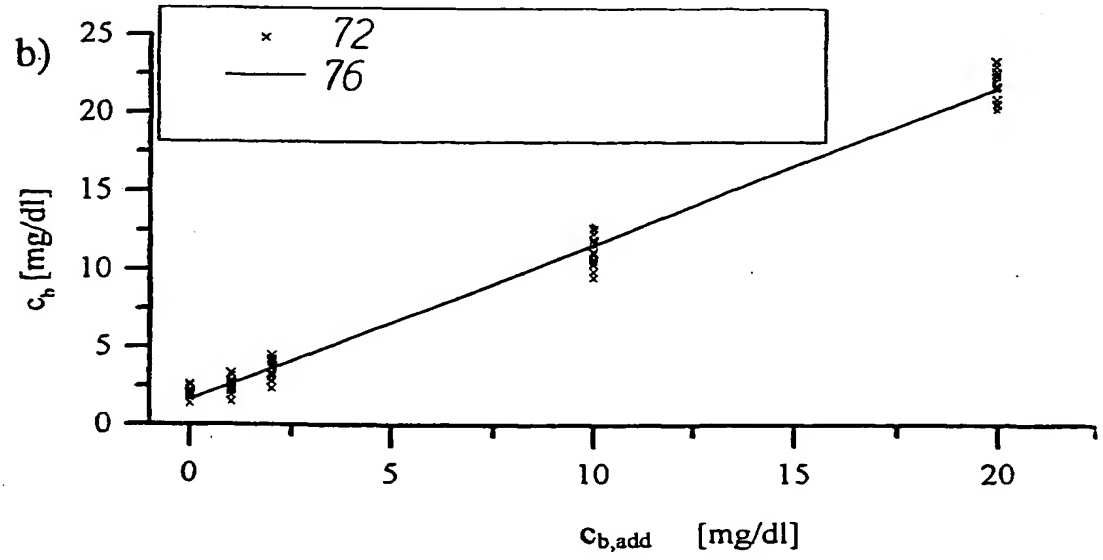

Fig. 9

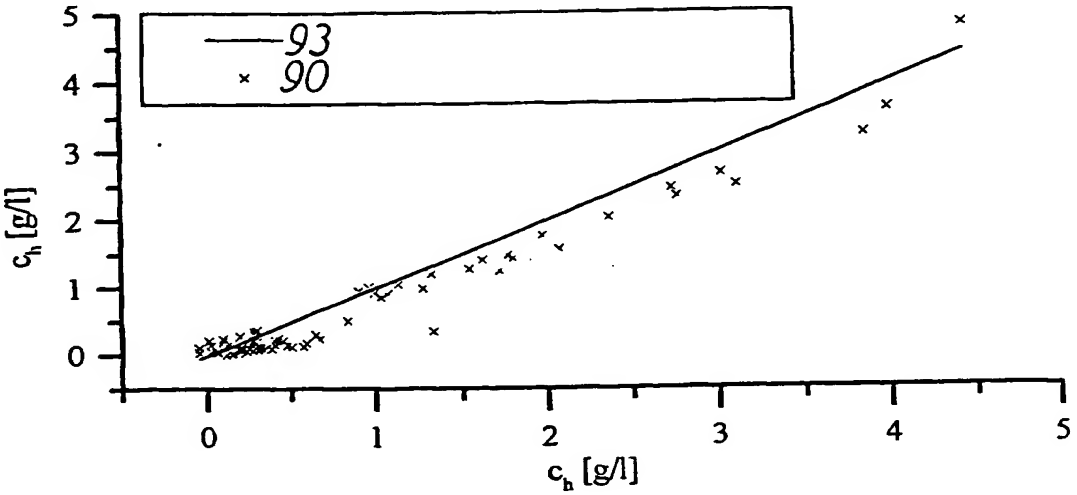

Fig. 10

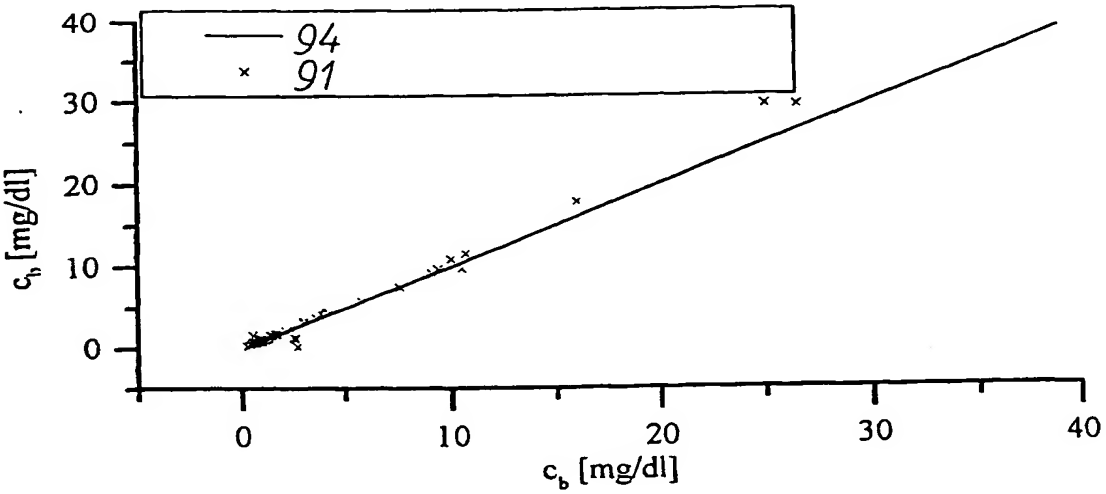

Fig. 11

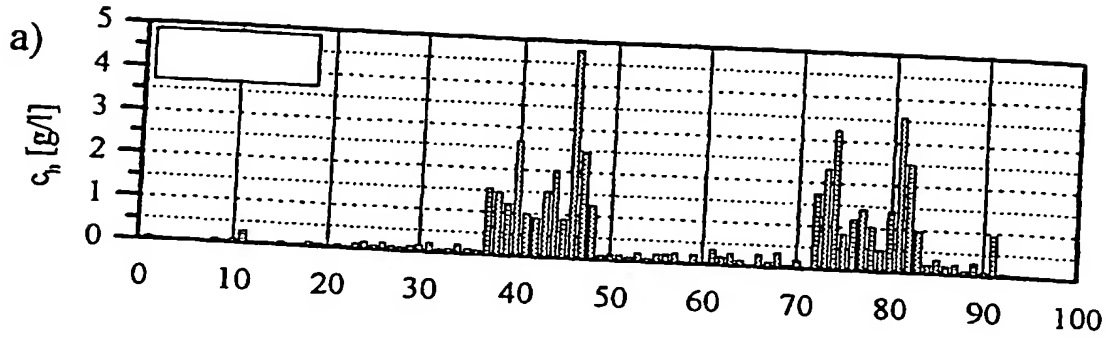

Fig. 12

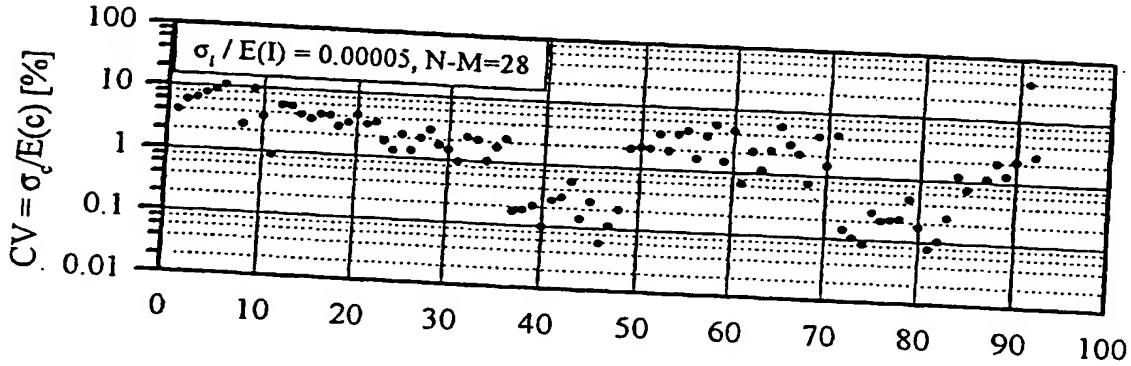

Fig. 13

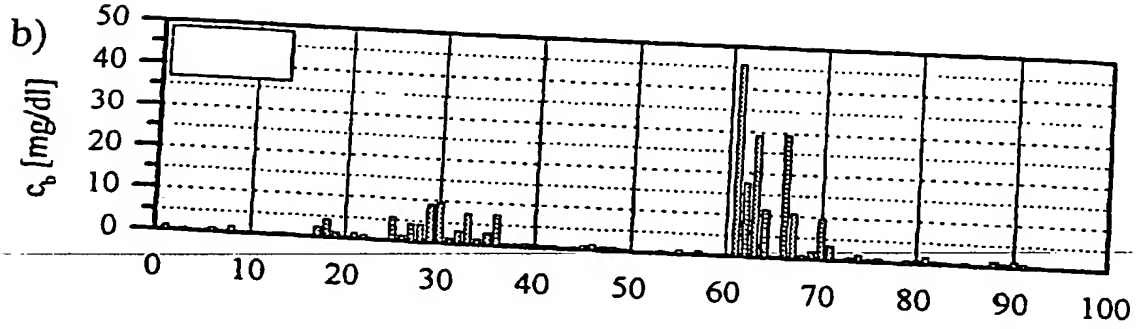

Fig. 14

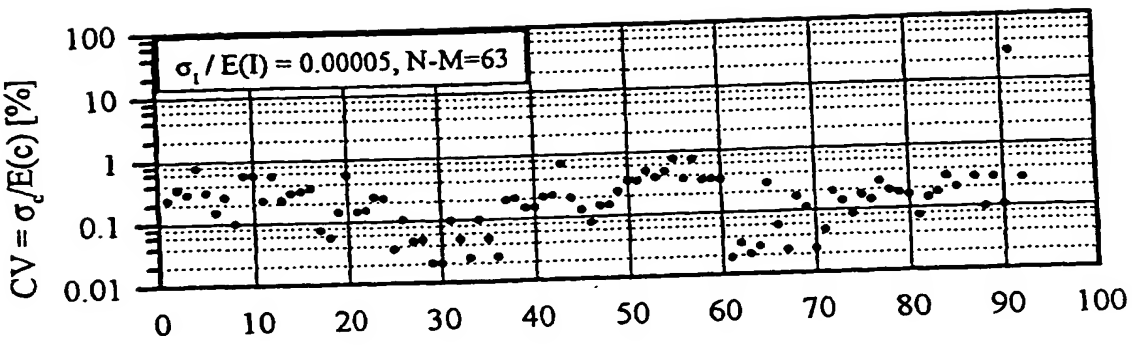
σ_t / E(I) = 0.00005, N-M=63
CV = σ_c/E(c) [%]
100
10
1
0.1
0.01
0
10
20
30
40
50
60
70
80
90
100

Fig. 15

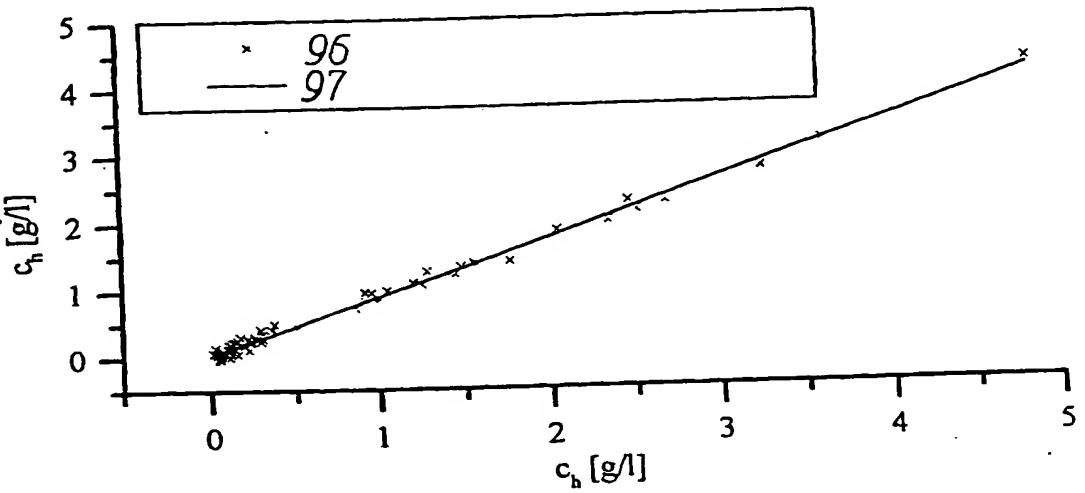
96
97
c_h [g/l]
c_h [g/l]
0
1
2
3
4
5

Fig. 16

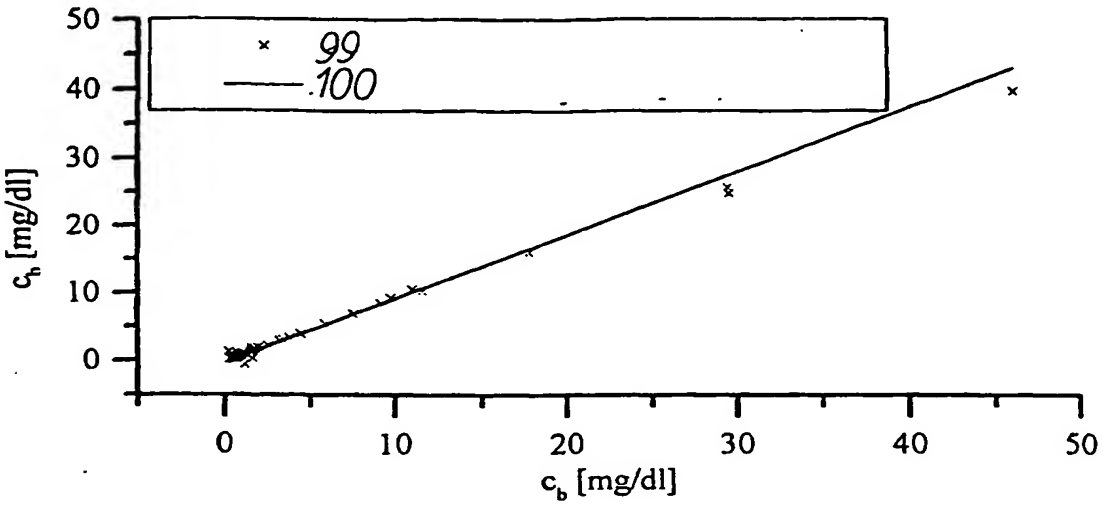
99
100
50
40
30
20
10
0
c_b [mg/dl]
0
10
20
30
40
50
c_b [mg/dl]

Fig. 17

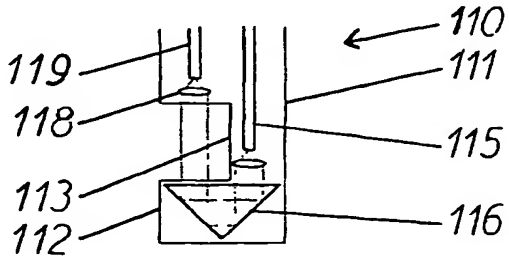
110
111
115
116
119
118
113
112

Fig. 18